Immunoglobulin-binding domains: Protein L from *Peptostreptococcus magnus*

N.G. Housden*, S. Harrison*, S.E. Roberts*, J.A. Beckingham*, M. Graille†, E. Stura† and M.G. Gore*[1]

*Division of Biochemistry and Molecular Biology, Institute of Biomolecular Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, U.K., and †Laboratoire de Structure des Protèines, DIEP, CEA, Centre d'Etudes de Saclay, Gif-sur-Yvette, F91191, France

Abstract

Protein L is a multidomain cell-wall protein isolated from *Peptostreptococcus magnus*. It belongs to a group of proteins that contain repeated domains that are able to bind to Igs without stimulating an immune response, the most characterized of this group being Protein A (*Staphylococcus aureus*) and Protein G (*Streptococcus*). Both of these proteins bind predominantly to the interface of C_H2-C_H3 heavy chains, while Protein L binds exclusively to the V_L domain of the κ-chain. The function of these proteins *in vivo* is not clear but it is thought that they enable the bacteria to evade the host's immune system. Two binding sites for κ-chain on a single Ig-binding domain from Protein L have recently been reported and we give evidence that one site has a 25–55-fold higher affinity for κ-chain than the second site.

Ig-binding proteins are expressed on the cell surface of many Gram-positive pathogenic bacteria and are able to bind to Igs in a non-antigenic manner [1], thus making them very valuable as diagnostic, therapeutic and preparative tools. The role of these proteins is almost certainly to help the bacteria adhere to wound surfaces and then evade the immune response by allowing host proteins to cover the bacterial cell surface. Two of the first such proteins to be recognized were Protein A, a cell-wall component of *Staphylococcus aureus*, and Protein G, a protein associated with the cell walls of certain *Streptococci* (for a review, see [1]). Both proteins have a high affinity for the interface between the C_H2 and C_H3 domains of IgG-Fc [2], but although their binding is competitive their interactions are not identical [3]. They also exhibit different levels of binding activity towards the different IgG subclasses and towards IgG from different animal species. More recently, attention has turned to a third type of Ig-binding protein. Protein L, expressed at the surface of approx. 10% of *Peptostreptococcus magnus* isolates (strains 312 and 3316), is an elongated molecule which has been shown to bind to the framework regions of the variable domain of κ light chains from any class of Ig [4]. Although it binds close to the antigen-binding site, this interaction does not affect the antigen–Ig interaction.

One common feature of Proteins A, G and L is that they all possess multiple copies of Ig-binding domains, each containing approx. 60–70 residues. Within each protein these domains are highly conserved, although some sequence differences exist that give rise to a range of binding affinities that causes Igs to be eluted from affinity columns in non-symmetrical peaks. Furthermore, the interactions are so strong that the pH at which the elution occurs is very low and thus these native proteins are not ideal ligands for affinity purification.

The structures of single Ig-binding domains from Protein A (SpA), Protein G (SpG) and Protein L (PpL) and their complexes have been obtained by X-ray crystallographic and NMR studies [5–9]. These have shown that, although SpA and SpG compete for similar binding sites on Fc, they have very different secondary structures. SpA consists of three α-helices [10] whereas SpG consists of two pairs of anti-parallel β-sheets connected by a single α-helix [11–14]. Helices 1 and 2 of SpA bind to Fc primarily via hydrophobic interactions and the interaction is further stabilized by four hydrogen bonds between the molecules [10]. SpG binds to Fc via interactions made by some residues on the helix and the loop region linking this to β-strand 3 [3,10]. In comparison with their strong Fc-binding ability, weak Fab binding has been observed for both proteins. Whereas SpA utilizes interactions between helices 2 and 3 to bind to a group of Igs encoded by the human V_{HIII} gene family [15], SpG offers interactions from β-strand 2 to bind weakly to the C_{H1} domain of Fab [6]. Despite their limited binding properties, such Ig-binding proteins have found worldwide use in the purification of antibodies, in immunoassays and in some therapies (to remove immune complexes from serum to minimize autoimmune disease and renal transplant rejection [16–20]).

PpL has a structure similar to that of SpG despite little sequence identity [21], the predominant difference being a shorter loop between β-strand 3 and the helix, which results in the angle of the α-helix to the β-sheet being 10% instead of the 40% seen in SpG. It is interesting that the loop region in SpG is responsible for Fc binding and therefore the shorter loop in PpL may well account for the inability of PpL to bind to Fc. Since the interaction between Protein L and Ig is with the light chain, there is no Ig-class restriction to its binding properties. It therefore offers the potential of

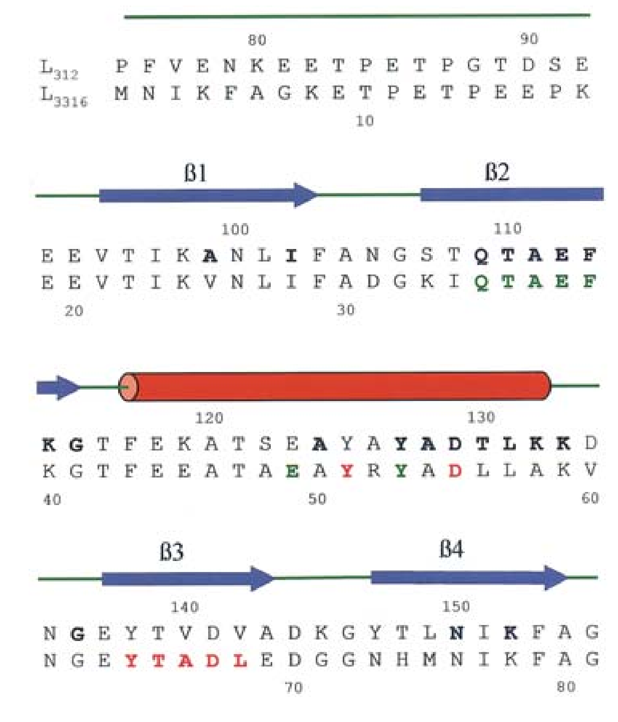
Key words: dissociation constant, immunoglobulin, mutant, sequence.

[1]To whom correspondence should be addressed (e-mail m.g.gore@soton.ac.uk).

©2003 Biochemical Society

Figure 1 | Sequence alignment of single domains from *P. magnus* strains 312 and 3316

The emboldened residues in the sequence of PpL from strain 312 are those shown by NMR studies to have major chemical shifts when in complex [4]. Those emboldened residues in the sequence of PpL from strain 3316 are those key residues that have been shown by X-ray crystallographic studies [26] to interact with human Fab at the classical site (green) and the non-classical site (magenta). Note that Arg-52 has been implicated in binding at both sites. The numbering used is that described in [29,30] for strains 312 and 3316 respectively.



being a universal Ig ligand. It also binds to scFv fragments which are comprised of V_H and the V_L domains linked by a short flexible peptide and contain an effective antigen-binding site. Their much-reduced size facilitates tissue penetration and minimizes immune response by the recipient and are therefore of current interest for immune therapy.

The two strains of *P. magnus* (312 and 3316) produce slightly different Protein L molecules. Strain 312 produces Protein L that contains five highly homologous Ig-binding domains [22], whereas strain 3316 produces a protein with four Ig-binding domains [23]. NMR studies [21,24,25] have shown that a domain from strain 312 interacts with the framework region of κ chains but no precise details of the contacts were obtained. The binding interaction elucidated by the NMR studies has been termed the 'classical binding interaction' [26] because of its similarity to the interaction between Protein G and the C_H1 domain of Fab [8] (Figure 1).

X-ray crystallographic studies [26,27] have recently determined the structure of PpL3316 in complexes with human and murine Fabs. In contrast with previous studies, these showed that two Fabs are able to bind to a single PpL

Table 1 | The K_d values, determined by ELISAs, for complexes formed between human polyclonal IgG and various mutant PpL domains

PpL mutant	Position of mutation: Site 1	Site 2	K_d (μM) ($\pm$S.D.; $n = 3$)
Wild-type			0.11 ± 0.02
F39W	✓		0.14 ± 0.03
E45Q	✓		0.11 ± 0.02
Y53F	✓		3.20 ± 0.2
Y53W	✓		5.93 ± 3.0
L57H	✓		2.80 ± 0.8
Y64W		✓	0.13 ± 0.02
D55AY64W		✓✓	0.21 ± 0.01

domain. The previously observed (classical) binding surface of PpL is large, involving 12 residues mainly from its β-strand 2 and the α-helix. Approx. 650 Å^2 of the surface area of each partner is buried upon complex formation at this site, i.e. a total of 1300 Å^2 of surface area is lost from the two components on complex formation. This interaction involves 13 residues from the κ-chain. Ten of these are within the framework region and the other three lie outside the hypervariable loops, thus not affecting antigen binding [26]. The complex at this site is stabilized by six hydrogen bonds; three lie between main chain atoms on β-strand 2 of PpL and the first β-strand of the V_L domain to form a β-zipper. Two others involve side chains of PpL residues (Tyr-53 and Glu-38) and one is formed between a Fab side chain and a backbone amide group of PpL. This classical binding site has a high affinity for κ_I, κ_{III} and κ_{IV} light chains but doesn't bind to κ_{II} or λ chains. The latter is due to sequence differences in κ_{II} and λ chains (e.g. a proline in position 12 of κ_{II}) that result in a changed backbone conformation that produce steric restrictions to the formation of the β-zipper within the classical binding site. The binding of Ig to this site has been characterized [28] using a number of different techniques including ELISA, fluorimetry (using placed Trp reporter groups on β-strand 2) and isothermal titration calorimetry. It has a K_a of approx. 10^7 M^{-1} [28], demonstrating that the affinity of the single domain is approx. 100-fold lower than that of the multiple domain native protein.

The binding interactions at the newly identified, non-classical site primarily involves 14 residues between residues 40 and 71 on the opposite side of PpL, i.e. β-strand 3 and part of the α-helix. The interaction is stabilized by six hydrogen and two salt bridges and a total surface area of 1400 Å^2 is buried when Fab binds to this site on PpL [26].

Mutations of residues implicated in site 2 (e.g. Y64W or a mutant bearing two substitutions in site 2, D55A/Y64W) have no discernible effect on the stability of the complex. Table 1 shows typical values determined by ELISA. Typical K_d values obtained for these mutant proteins with human polyclonal IgG at neutral pH were around 100–200 nM (see Table 1), similar to that for the wild-type protein. However,

©2003 Biochemical Society

mutations of residues in site 1, such as Y53F and L57H, result in K_d values for their complexes with human IgG of approx. 3–6 μM. These studies therefore suggest that despite site 2 having more interactions with Fab than site 1, the affinity of this site is approx. 25–55-fold lower than that at site 1 [26].

References

1 Goward, C., Scawen, M., Murphy, J. and Atkinson, T. (1993) Trends Biochem. Sci. **18**, 136–140
2 Boyle, M.D.P. (1990) Bacterial Immunoglobulin-Binding Proteins, Academic Press, San Diego, CA
3 Sauer-Eriksson, A.E., Kleywegt, G.J., Uhlén, M. and Jones, T.A. (1995) Structure **3**, 265–278
4 Enokizono, J., Wikström, M., Sjöbring, U., Björck, L., Forsén, S., Arata, Y., Kato, Y. and Shimada, I. (1997) J. Mol. Biol. **270**, 8–13
5 Gouda, H., Torigoe, H., Saito, A., Sato, M., Arata, Y. and Schimada, I. (1992) Biochemistry **31**, 9665–9672
6 Derrick, J.P. and Wigley, D.B. (1992) Nature (London) **359**, 752–754
7 Starovasnik, M.A., O'Connell, M.P., Fairbrother, W.J. and Kelley, R.F. (1999) Protein Sci. **8**, 1423–1431
8 Lian, L. (1994) Struct. Biol. **1**, 355–357
9 Boyle, M.D.P. and Reis, K.J. (1987) Bio/Technology **5**, 697–703
10 Deisenhofer, J. (1981) Biochemistry **20**, 2361–2370
11 Achari, A., Hale, S.P., Howard, A.J., Clore, G.M., Gronenborn, A.M., Hardman, K.D. and Whitlow, M. (1992) Biochemistry **31**, 10449–10457
12 Lian, L., Yang, J.C., Derrick, J.P., Sutcliffe, M.J., Roberts, G.C.K., Murphy, J.P., Goward, C.R. and Atkinson, T. (1991) Biochemistry **30**, 5335–5340
13 Lian, L., Derrick, J.P., Sutcliffe, M.J., Yang, J.C. and Roberts, G.C.K. (1992) J. Mol. Biol. **228**, 1219–1234
14 Gronenborn, A.M., Filpula, D.R., Essig, N.Z., Achari, A., Whitlow, M., Winfield, P.T. and Clore, G.M. (1991) Science **253**, 657–671
15 Sasso, E.H., Silverman, G.J. and Mannik, M. (1991) J. Immunol. **147**, 1877–1883
16 Ey, P.L., Prowse, S.J. and Jenkin, C.R. (1978) Immunochemistry **15**, 429–436
17 Hjelm, H., Hjelm, K. and Sjöquist, J. (1972) FEBS Lett. **28**, 73–76
18 Stingl, G., Wolff-Schriener, E.C. and Pilcher, W.J. (1977) Nature (London) **268**, 245–246
19 Bansal, S.C., Bansal, B.R., Rhoads, J.E., Cooper, D.R., Boland, J.P. and Mark, R. (1978) Int. J. Artificial Organs **1**, 94–103
20 Barocci, S. and Nocera, A. (1993) Transplant Int. **6**, 29–33
21 Wikström, M., Drakenberg, T., Forsén, S., Sjöbring, U. and Björck, L. (1994) Biochemistry **33**, 14011–14017
22 Kastern, W., Sjöbring, U. and Björck, L. (1992) J. Biol. Chem. **267**, 12820–12825
23 Murphy, J.P., Duggleby, C.J., Atkinson, M.A., Trowen, A.R., Atkinson, T. and Goward, C.R. (1994) Mol. Microbiol. **12**, 911–920
24 Wikström, M., Sjöbring, U., Drakenberg, T., Forsén, S. and Björck, L. (1995) J. Mol. Biol. **250**, 128–133
25 Wikström, M., Forsén, S. and Drakenberg, T. (1996) Eur. J. Biochem. **235**, 543–548
26 Graille, M., Stura, E.A., Housden, N.G., Beckingham, J.A., Bottomley, S.P., Beale, D., Taussig, M.J., Sutton, B.J., Gore, M.G. and Charbonnier, J.B. (2001) Structure **9**, 679–687
27 Graille, M., Harrison, S., Crump, M.P., Findlow, S.C., Housden, N.G., Muller, B.H., Battail-Poirot, N., Sibaï, G., Sutton, B.J., Jolivet-Reynaud, C. et al. (2002) J. Biol. Chem. **277**, 47500–47506
28 Beckingham, J.A., Bottomley, S.P., Hinton, R., Sutton, B.J. and Gore, M.G. (1999) Biochem. J. **340**, 193–199
29 Wikström, M., Sjöbring, U., Kastern, W., Björck, L., Drakenberg, T. and Forsén, S. (1993) Biochemistry **32**, 3381–3386
30 Bottomley, S.P., Beckingham, J.A., Murphy, J.P., Atkinson, M., Atkinson, T., Hinton, R.J. and Gore, M.G. (1995) Bioseparation **5**, 359–367

Received 23 January 2003

©2003 Biochemical Society